Quantitative Vaginal Culture

Collect a vaginal sample with the Culture Swab Plus™ Amies gel double swab from BBL™ with charcoal (Blomgren E, Gandhi B, Ratnam S, Stoner KA). Transport swabs at room temperature to laboratory within 24 hours. Agitate both swabs in 1.5ml of Bacto™ Proteose Peptone Broth no.3 (PPB) (Dunkleberg WE) and vortex to make an approximate 1:10 dilution (Hillier SL). Transfer 10ul of the first dilution serially into 1ml of PPB to make 10^{-3}, 10^{-5}, and 10^{-7} dilutions vortexing between each dilution. Inoculate Columbia blood agar with 5% sheep blood (CBA), Rogosa agar, and Human Blood Tween agar (HBT) (Totten PA) with 100ul of each dilution. Use a loop and streak for isolation on the 10^{-1} dilution. Use a spreader bar for the last three dilutions. Incubate Rogosa plates for 48 hours in anaerobic conditions at 37°C. Incubate HBT and CBA for 48 hours in 3-5% CO_2 for 48 hours.

Growth from the four different dilutions will be examined on all three types of agar. The plates that best represents all colony types and still have well isolated colonies will be used for both quantification and identification. In order to quantify an organism the number of colonies must be counted on the agar plate of the dilution chosen. If the 10^{-1} dilution is chosen, then the number of colonies counted must be multiplied by 10^{2} to get colony forming units per ml (cfu/ml). If the 10^{-3}, 10^{-5}, or 10^{-7} dilution is chosen, then the number of colonies counted must be multiplied by 10^{4}, 10^{6}, or 10^{8} respectively. For example, 13 colonies of Lactobacilli on the 10^{-5} agar plate would be equal to 13×10^{6} cfu/ml.

All isolates will be archived for future studies at -70°C in Litmus Milk with 20% glycerol.

Lactobacillus Isolation and Presumptive Identification and H_2O_2 Production

Potential Lactobacillus colonies will be Gram stained to verify that large gram positive rods are present, and that colonies are catalase negative. Catalase negative, Gram positive rods will be subcultured onto a Columbia blood agar plate. The blood agar plates will be incubated for 48 hours, in the same manner as described before. After the 48 hour incubation, the plates are to be checked for purity. If the culture is mixed, the culture will be restreaked from a pure isolated colony onto a blood agar plate, and incubated in the manner as described before. This procedure will be repeated until a pure culture is obtained.

The Rogosa plate is also used for isolating Lactobacillus species. Rogosa is a selective media that is useful for isolating Lactobacillus in small quantities and when other organisms overgrow the Columbia blood agar. Not all species of Lactobacillus will grow on Rogosa agar therefore Rogosa should not be used as the only media for the isolation of Lactobacillus. Isolation of Lactobacillus from the Rogosa is done so in the same manner as described above.

Lactobacillus isolates will be tested for production of hydrogen peroxide using Tetra methyl benzidine (TMB) agar. The TMB agar will be inoculated with the test organisms and incubated anaerobically for 48 hours. Known positive and negative hydrogen peroxide producing Lactobacilli will be included as controls. The plate will then be exposed to an aerobic environment for 30 minutes and examined for positive or negative results. A blue color where the colony is growing indicates a positive result. No color change indicates a negative result.

Group B Streptococcus Isolation and Identification

Examine plate for large, white, semi-opaque, beta to slight beta (sometimes non-beta) hemolytic colonies. Subculture suspect colonies to another CBA plate and incubate for 24 hours at 37°C in 3-5% CO_2. Check subculture for purity. Group B Streptococcus should be catalase negative, Gram positive cocci in pairs and chains, and CAMP test positive.

Gardnerella vaginalis Isolation and Identification

Gardnerella vaginalis-like colonies, small and hemolytic on HBT, and non-hemolytic on CBA, will be subcultured to CBA. A presumptive identification will be done using gram stain and the catalase test (Catlin BW). Those colonies that are catalase negative and gram-variable, small pleomorphic rods will be tested with the MicroScan® Haemophilus-Neisseria Identification panel (HNID®) for confirmation (Janda WM).